INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Photocatalytic Dye Degradation and Bacteriostatic Efficacy of Myco Synthesized Copper Nanoparticles by new Isolate *Lentinus squarrosulus (Mont.)* from Dead Trunk of *Nerium odourum*

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OPEN ACCESS

Received: 04-02-2023
Accepted: 31-05-2023
Published: 24-06-2023

Citation: Amirtham PJM, Shenbhagaraman R, Siva R (2023) Photocatalytic Dye Degradation and Bacteriostatic Efficacy of Myco Synthesized Copper Nanoparticles by new Isolate *Lentinus squarrosulus (Mont.)* from Dead Trunk of *Nerium odourum*. Indian Journal of Science and Technology 16(24): 1802-1809. https://doi.org/10.17485/IJST/v16i24.252

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To investigate the antibacterial activity and photo-catalytic efficacy of CuNPs synthesized from *Lentinus squarrosulus* used for the degradation of commercial textile dyes. **Methods:** The Copper nanoparticles (CuNPs) were biosynthesized using *L. squarrosulus*, a new fungal isolate from the dead trunk of *N. odorum*. The production of copper nanoparticles using mycoextract was simple and environmentally friendly, yielding stable copper nanoparticles in a variety of shapes. Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), and EDAX analysis have all been used to establish that CuNPs were successfully formed. **Findings:** The research findings of the present study show that absorption spectra around 300 to 350 nm in UV-vis spectra followed by the presence of FT-IR peaks at 3680cm^{-1} and 3856.7cm^{-1} of proteins, carbohydrates, flavonoids, and tannins confirmed the myco-synthesis of copper nanoparticles using *L. squarrosulus*. Additionally, scanning electron microscopy examination of their external morphology reveals the existence of CuNPs with an average particle size of about 200 nm each, which are the primary spherical nanoparticles. Energy-dispersive x-ray (EDX) analysis was performed to further investigate the configuration of copper nanoparticles and it was found that, pure copper (03.51 percent) was present in CuNPs. The produced CuNPs were assessed for their antimicrobial activity and the results show that they were highly effective against *Enterobacter aerogenes* MTCC 2823 and *Streptococcus mutans* MTCC 497, while *Escherichia coli* MTCC 739 and *Enterococcus faecalis* MTCC 439 showed moderate antibacterial activity. Further, the photocatalytic dye degradation potentials of myco-synthesized CuNPs showed higher degradation efficiency in fast green dye, moderate degradation rate in Congo red dye and a very low degradation rate in the brilliant blue dye. **Novelty :** Preparation of mycogenic copper nanoparticles

from *L. squarrosulus* and their photocatalytic degradation of organic dyes are the novel part in this study as there is no remarkable study in specific CuNPs.

Keywords: Lentinus Squarrosulus; Antibacterial; Green Synthesis; Photocatalytic; Dye Degradation; Copper Nanoparticles

1 Introduction

The catalytic, optical, and electrical capabilities of copper nanoparticles have drawn a lot of attention. Therefore, due to its low cost and high conductivity, copper is mostly chosen for the nanoparticle synthesis process[(1)]. The use of copper nanoparticles as antibacterial, anticancer, antioxidant, anti-inflammatory and antihepatotoxic agents is widespread. At the moment, copper nanoparticles are utilized as an alternative feed ingredient in animal feed, particularly in the diet of chicken[(2)]. In the lab, fungi are easy to work with and release a lot of enzymes[(3)]. However, relatively few studies on the myco-synthesis of CuO NPs have been documented. Three different Penicillium species, *P. waksmanii, P. citrinum, and P. aurantiogriseum* were used in a biological method to produce CuO NPs from copper sulphate (CuSO4). Additionally, it was confirmed that the white-rot fungus *Stereum hirsutum* can be used in the production of CuO NPs[(4)]. Due to the availability of a biological property, nanoparticle production using mushroom extracts has drawn the most attention[(5)].

A genus of wood rotters known as "white rot fungus" emits extracellular enzymes, primarily lignin peroxidase, manganese peroxidase, and laccase, which breaks down lignin and due to this potential it can be utilised as an excellent resource that can be used in the bioremediation of pollutants[(6)].

Use of myco-remediation to mineralize synthetic dyes from textile effluents is a useful and environmentally beneficial method[(7)]. Industries like those in the food processing, pharmaceutical, textile, leather, chemical, dyestuff and dyeing sectors release a lot of effluents, including various types of processed dyes[(8)]. The textile industry is one of the biggest dischargers of dyes and colours from the factories that have a direct impact by disturbing the soil and water ecosystem. The environmental concerns caused by textile dye are many and the disruption of ecosystem and contamination of soil and water sources are the prime issues.

Eutrophication is brought on by the buildup of organic pollutants and textile dyes, which also lowers the capacity for oxygenation and severely harms aquatic microorganisms[(8)]. Owing to their huge surface area, Nps (Nanoparticles) are now attracting a lot of interest in the detoxification of dangerous dyes[(9)], with a particular emphasis on the photocatalytic degradation of dyes utilizing metallic nanoparticles[(10)]. The advantages of myco-synthesized CuNPs possess biocompatibility properties with less toxicity.

Lentinus squarrosulus (Mont.), a basidiomycetes fungus - is well- known for its nutritional factors and its ethno-medicinal values in the treatment of ulcer, anaemia and other ailments[(11)]. They have been reported to have anti-tumor[(12)], antidiabetic[(13)], antioxidant activities and toxicological properties[(11)]. Till now synthesis of copper oxide nanoparticle from *L. squarrosulus* extract is not yet reported. However, it differs from other fungi CuNPs as it was prepared from a white rot fungus with the potentiality for bioremediation.

The present investigation highlights the mycogenic preparation of CuNPs by utilizing the white rot fungi *L. squarrosulus*. Further, the characterization of CuNps, its photocatalytic degradation and antibacterial activity was studied.

2 Methodology

2.1 Mushroom sample collection and identification

The *L. squarrosulus* was collected from the dead trunk of N. odourum trees of the Tambaram region (12.92° N, 80.10° E). The mushroom was identified by DNA sequencing. The dried fruit body of the sample was stored in the culture collection Centre in Shrimathi Devkunvar Nanalal Bhatt Vaishnav College, Chrompet, Chennai.

2.2 Preparation of Mushroom Extracts

The *L. squarrosulus* sample was used for the extraction of copper nanoparticles. Using a laboratory mixer, the shade-dried *L. squarrosulus* was powdered. From the dried powder sample, a 10mg aliquot was solubilized in 100ml double distilled water and the suspension was boiled at 60°C for 10mins (Figure 1). The resulting extract was cooled at room temperature and sieved through a Whatmann filter No. 1 filter paper and the filtrate were preserved at 4°C till usage.

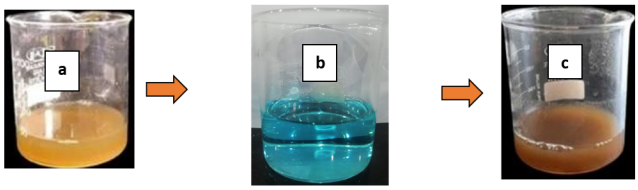

Fig 1. Visual representation of synthesis of copper nanoparticles from *Lentinus squarrosulus* **.** (a) Aqueous extract of Mushroom**,** (b) Copper sulphate, (c) CuNPs Suspension

2.3 Preparation, Synthesis and Characterization of CuNPS

The mushroom extract was combined with 5mM of CuSO4 in 1:2 ratio and let to stand until an additional colour change occurred. The colour change serves as an indicator to know the synthesis of nanoparticles, when compared to the control solution.The color variations in the solution showed the synthesis of Copper Nanoparticles. After the reaction time, the sample containing CuNPs was centrifuged in room temperature at 15,000 rpm, for 20 mins. The supernatant was removed after centrifugation and washed twice with sterile deionized water. Then, these myco-synthesized CuNPs were lyophilized and used for further physical characterization. The synthesis of CuNPs in the sample was further confirmed by UV-Visible spectrometer (Elico SL159 UV Vis Spectrophotometer). The samples were subjected to FT-IR spectroscopy (Perkin Elmer FT-IR Spectroscopy) in the wave number between 400 cm^{-1} and 4000 cm^{-1}. Scanning electron microscopy was used to analyze the surface morphology of the CuNPs (Carl Zeiss EVO 18, Germany). To validate the composition analysis of the produced CuNPs, the energy dispersive X-ray (EDX) analysis was also performed.

2.4 Antibacterial activity of synthesized CuNPs

Cultures were obtained from MTCC (Microbial Type Culture Collection and Gene Bank), Chandigarh, India. In this study both the Gram- negative bacteria -Escherichia coli MTCC 739, *Enterobacter aerogenes* MTCC 2823 and Gram-positive bacteria - *Streptococcus mutans* MTCC 497, *Enterococcus faecalis* MTCC 439 were used to assess the antibacterial potential of the myco-synthesized CuNPs. The standard disc diffusion method was performed to assess the antibacterial effect of myco-synthesized CuNPs[14,15]. The microbial inoculum culture was prepared in Luria Bertani broth at 37°C for 24 h. Bacterial suspensions were spread on a nutrient agar medium by swabbing technique. A set of two concentrations (25μL and 50μL) of CuNPs solution was suspended on to the surface of the disc, dried, and then used for the study. Over the bacterial culture lawn, the positive control antibiotic ampicillin disc was placed, the discs of two different concentration were placed over the bacterial lawn culture. All the cultured plates were kept at 37°C for 24h in bacteriological incubator. A measuring ruler was used to calculate the millimeter value of the zone of inhibition (ZOI) surrounding the discs.

2.5 Photocatalytic reduction of Dyes

The photocatalytic activity of CuNPs synthesized from mushroom was assessed for the degradation of Congo red dye, fast green dye,and brilliant blue dye. As a stock solution, 1000 mL of deionized water was mixed with 10 mg each of Congo red dye, quick

green dye, and brilliant blue. About 20mg of biosynthesized copper nanoparticles was added to each dye solution (Congo red, Brilliant Blue, and Fast Green) containing 100 ml along with control (without CuNPs). To initiate photocatalytic degradation, the suspension was exposed to UV radiation as follows: the reaction mixture was stirred continuously using a magnetic stirrer, and periodically (0min, 30 min, 60 min, 75 min, and 90min) the absorption was measured by taking 3ml aliquots of 3ml of suspension was withdrawn centrifuged and analyzed using UV–Vis spectroscopy. To evaluate photocatalytic dye degradation, the characteristic peak at 633nm of the Congo red dye, fast green dye, and brilliant blue dye was recorded and their degradation efficiency was calculated.

3 Results and Discussion

As mentioned previously, the mushroom-synthesized nanoparticles are more stable and non-toxic compared to other microorganism-assisted extracts of plants synthesized nanoparticles[16]. The right combination of temperature and time is crucial for the production of nanoparticles. It is commonly known that mushrooms are a high-protein food with a protein content of 75%. The protein content of the aqueous mushroom extract is high and tryptophan, glutamic acid, lysine, and other amino acids as well as enzymes like laccase are readily available. Among other bioactive compounds, mushroom extracts are a great source of riboflavin. According to recent research, copper nanoparticles are formed by the flavoproteins found in mushroom extracts[17]. In a similar study a team of researchers discovered that pine mushroom flavoproteins are essential for the production of silver nanoparticles[18].

3.1 Characterization of myco-synthesized CuNPs

3.1.1 UV-Vis Spectroscopy and FTIR analysis

Strong absorption between 300 and 350 nm in the UV-vis spectra suggests the production of CuNPs (Figure 2 a). The CuNPs were examined through FTIR in the 400cm-^{1} to 4000cm-^{1}range for the occurrence of functional groups surrounding them. Various functional groups are represented by the FT-IR spectra of the myco-synthesized CuNPs in Figure 2 b. Minor shifts in bands were detected when mycelia extract was flooded with Cu2+ ions, which could be due to interaction between the Cu2+ ions and functional groups present in the extract. FTIR spectrum of myco- synthesized CuNPs, the main peaks at 1398.6 cm^{-1}, 1512.4 cm^{-1}, 2038.6 cm^{-1}, 2348.8 cm^{-1}, 3220.5cm^{-1}, 3680 cm^{-1} and 3856 cm^{-1} are present. All transmittance peak indicates some functional group present in the myco-synthesized nanoparticles. Peaks present at 1398.6 cm^{-1}, 1233.6cm^{-1}, and 1512.4cm^{-1}correspond to lignin, ester linkages, cellulose, and amide I group respectively. Band present at 3680$cm^{-1}$and 3856.7cm^{-1}corresponds to proteins, tannins, flavonoids, and carbohydrates. The incidence of SPR absorption reflects the size of the nanoparticles[19]. The peak at 319 nm in the UV spectrum was visible in the surface plasmon resonance of an aqueous solution of copper nanoparticles produced by *Pleurotus ostreatus*. These results demonstrate unequivocally that the produced molecules are CuNPs[20]. In recent work, Ghareib et al. produced Aspergillus niger-derived CuNPs and verified them using surface plasmon resonance at 335 nm[4].

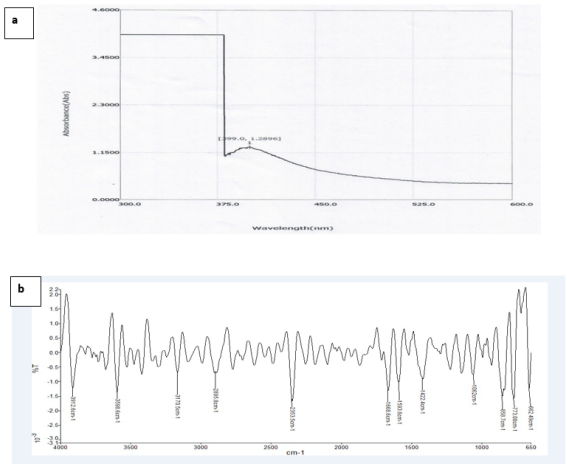

Fig 2. Spectral analysis of myco-synthesized copper nanoparticles (a) UV-Visible spectra (b) FT-IR spectrum

3.1.2 SEM Analysis of CuNPs nanoparticles

SEM was used to study the surface morphology of the size and shape and revealed the existence of copper nanoparticles (Figure 3). The Cu nanoparticles were found to have aggregated, and their average size is between 200 and 300 nm, according to SEM. The majority of the nanocopper particles are spherical, and there are also numerous aggregates of copper oxide nanoparticles, some of which exhibit nanoparticles with an ambiguous shape. SEM study of the size distribution showed that the CuNPs produced by *L. squarrosulus* have a pattern especially identical to the CuNPs produced under ideal conditions by *A. niger*, with a size of 500 nm [21].

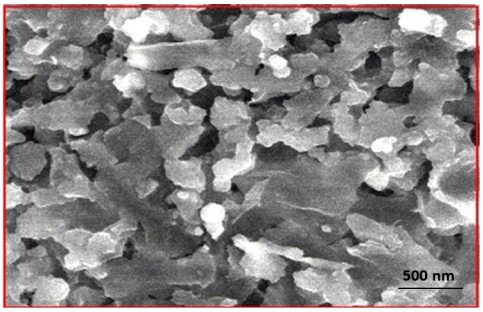

Fig 3. Scanning electron microscopic images of copper nanoparticles synthesized using *Lentinus squarrosulus*

3.1.3 EDX analysis

By using energy-dispersive X-ray (EDX) analysis, the composition of copper nanoparticles was further investigated. The EDX pattern of CuNPs derived from the mushroom extract is shown in Figure 4 and Table 1 , which suggests the presence of Cu and a significant amount of oxygen. Analysis using energy dispersive x-ray spectroscopy (EDX) demonstrated that CuNPs contained pure copper (03.51%). Peaks in the myco-synthesized CuNPs' EDAX analysis indicate the presence of copper.

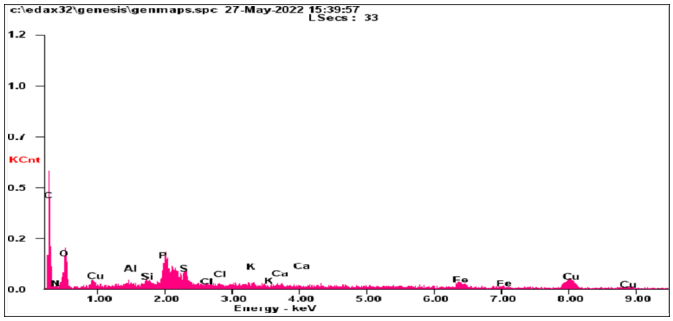

Fig 4. EDX spectrum of mycosynthesized copper nanoparticles

Table 1. Composition of Copper Nanoparticle synthesized from *Lentinus squarrosulus*

Element	Wt%	At%
CK	61.35	**70.55**
NK	06.43	**06.34**
OK	23.40	**20.20**
AlK	00.26	**00.13**
SiK	00.31	**00.15**
PK	02.24	**01.00**
SK	01.14	**00.49**
ClK	00.09	**00.04**
KK	00.15	**00.05**
CaK	00.09	**00.03**

Continued on next page

Table 1 continued

FeK	01.04	**00.26**
CuK	03.51	**00.76**
Matrix	**Correction**	**ZAF**

3.2 Antimicrobial activity of myco-syntheiszed CuNPs

Different concentrations of CuNPs were tested for their antibacterial efficacy against the pathogenic strains of *S. mutans* MTCC 497 (+ve), *E. faecalis* MTCC 439(+ve), *E. coli* MTCC 739 (-ve), and *E. aerogenes* MTCC 2823(-ve). The positive control, ampicillin was used in this study to evaluate the antibacterial effects of CuNPs. For two concentrations of CuNPs, the measured zone of inhibition in diameter (mm) around the discs were summarized in (Figure 5). The diameter of the zone of inhibition provides information regarding the degree of microbial sensitivity to CuNPs. The CuNPs showed very strong antibacterial activity against *Enterobacter aerogenes* MTCC 2823 and *S. mutans* MTCC 497, but exhibited moderate antibacterial activity against *Escherichia coli* MTCC 739 and *E. faecalis* MTCC 439. Therefore, myco-synthesized CuNPs could inhibit the growth of gram-positive pathogens. Studies of a similar nature using *A. niger* produced nanoparticles revealed potential zone development against *K. pneumoniae, S. aureus, E. coli, B. subtilis and M. luteus* respectively [21]. In a similar study $MgFeO_4$ nano particles shows good antimicrobial action against gram-positive bacteria *S. aureus* and gram-negative bacteria *E.coli* [22]. CuNPs have a well-established antibacterial activity, and the pharmaceutical sector frequently uses them in applications including the prevention of infections [23].

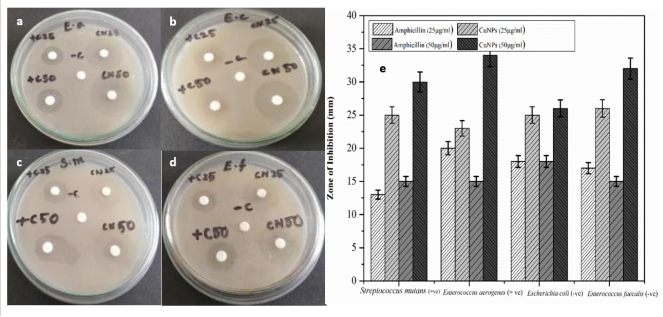

Fig 5. Antibacterial activity of copper nanoparticles synthesized from *Lentinus squarrosulus*.(a) *Enterobacter aerogens* MTCC 2823, (b) *Escherichia coli* MTCC 739, (c)*Streptococcus mutans* MTCC 497, (d) *Enterococcus faecalis* MTCC 439(e) Graphical representation of zone of inhibition (mm)

3.3 Photocatalytic activity

Photocatalytic actions of CuNPs have been observed by execution of degradation of different dyes such as Congored, fast green, and brilliant blue under UV light exposure. In UV-Vis spectroscopy, Congo red dye typically has an absorption peak at 498 nm, fast green at 619 nm, and bright blue solutions at 465 nm. The degradation of the dye solution was visualized by a decrease in the intensity of the representative absorbance maximum, as represented in Figure 6 (a,b,c). According to Figure 6 (d), the myco-synthesized CuNPs showed higher degradation efficiency in the fast green dye, followed by intermediate degradation efficiency in congo red dye, and significantly lower degradation efficiency in the brilliant blue dye. Photodegradation is affected by several basic parameters, including, quantity of photocatalyst, concentration of substrate, surface area of photocatalyst, structure of photocatalyst, light intensity, solution pH, temperature, dissolve oxygen, doping of metal ions and nonmetals and substrate [24]. Several scientists have studied the photodegradation of organic compounds and concluded the ideal conditions for the photo catalytic disintegration of organic compounds [25]. Recent reports showed that silver and copper nanoparticles synthesized from *Centella asiatica* showed a similar rate of degradation of dyes [26]. CuO nanoparticles from Diospyros montana's aqueous leaf extract have been shown to have photocatalytic activity, according to Siddiqi et al [27]. The photocatalytic activity of Seriphidium oliverianum's CuO NPs demonstrated high potential for the degradation of water-soluble commercial dyes, including methyl green (MG) and methyl orange (MO), according to further research by Aroob et al. [28]. According to reports, unique biomolecules in mushrooms, including proteins and other nutrients are major combinations responsible for the formation of copper particles. Thus, myco-extracted nanoparticles would be a better option for biomedical and biodegradation applications.

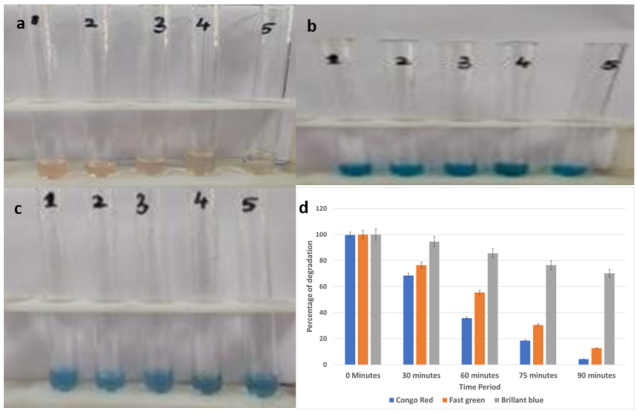

Fig 6. Photocatalytic degradation of dyes by CuNPs from *Lentinus squarrosulus* (a) Congo red,(b) Fast green, (c) Brilliant blue (d) Degradation potentials of CuNPs against three dyes

4 Conclusion

A simple and eco-friendly myco-synthesis method was used for copper nanoparticles from *Lentinus squarrosulus*. The good formation of nanoparticle copper nanoparticles was categorized by SPR band positioning and was demonstrated using UV-Vis Spectra. The synthesized nanoparticles were flakes of spherical agglomeration having high potential in the degradation of azo dye Congo red and triarylmethane dye fast green within hours after treatment. Further, it also showed potential antibacterial effects against *Enterobacter aerogenes* and *Streptococcus mutans*, making it a novel dye degradation biomaterial with disinfection properties.

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